

## **Supplementary Data**

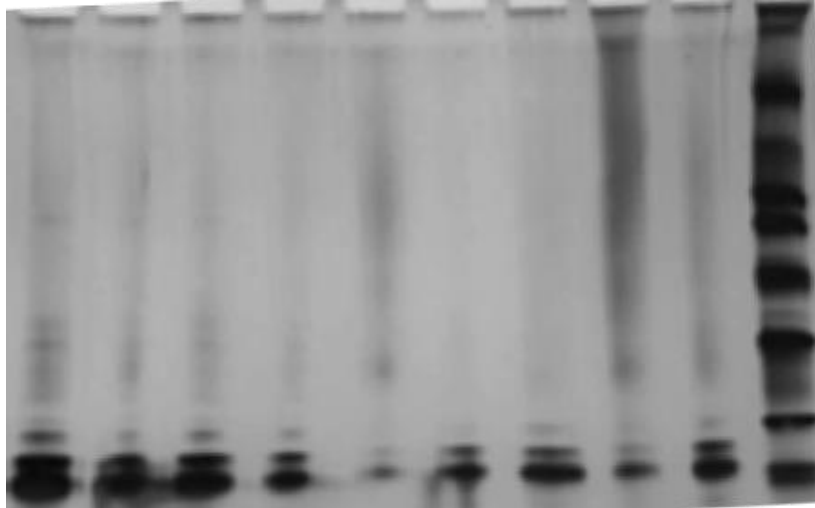
### **Cross-linking of lens crystallin proteins induced by tryptophan metabolites and metal ions: implications for cataract development**

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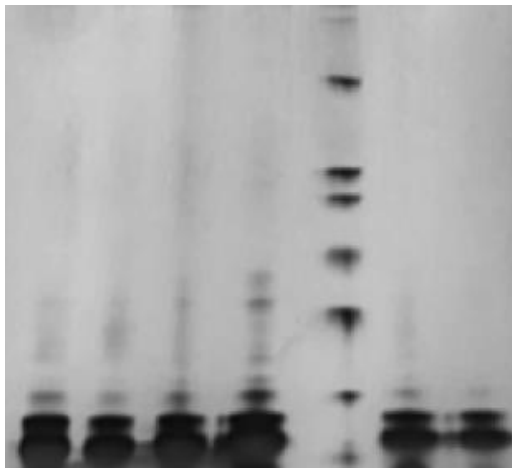
BLP and 3OHKyn present in all lanes									
Cu(II)	-	+	-	-	+	-	-	+	-
Fe(III)	-	-	+	-	-	+	-	-	+
1 h incubation	+	+	+	-	-	-	-	-	-
28 h incubation	-	-	-	+	+	+	-	-	-
5 days incubation	-	-	-	-	-	-	+	+	+

**Supplementary Figure 1** Extent of protein cross-linking (as detected by SDS-PAGE gels with silver staining) over time on incubation of mixtures containing 3OHKyn (10  $\mu$ M) and BLP (ca. 6  $\mu$ M) (present in all lanes except molecular mass marker lane without or with added Cu(II) (10  $\mu$ M) or Fe(III) (10  $\mu$ M), as indicated by the grid, under aerobic conditions in PBS at 37 °C for 1 h, 28 h or 5 days in the dark. The lane on the far right contained molecular mass markers.



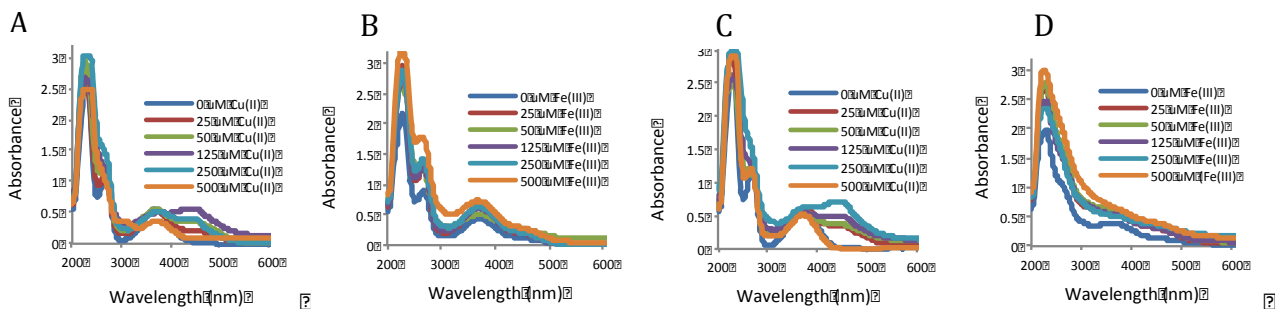
BLP	+	+	+	-
3OHKyn	+	+	+	-
3OHKyn-BLP	-	-	-	+
Cu(II)	-	+	-	-
Fe(III)	-	-	+	-

**Supplementary Figure 2** Comparison of the extent of protein cross-linking (as detected by SDS-PAGE gels with silver staining) induced on incubation of BLP (ca. 6  $\mu$ M in protein) with Cu(II) (10  $\mu$ M), or Fe(III) (10  $\mu$ M) or 3OHKyn-bound to BLP (ca. 6  $\mu$ M in protein), as indicated by the grid, under aerobic conditions in PBS at 37 °C for 5 days in the dark.



BLP	+	+	+	-	-	-	-
Kyn-BLP	-	-	-	+	-	+	+
Cu(II)	-	+	-	-	-	+	-
Fe(III)	-	-	+	-	-	-	+
Standards	-	-	-	-	+	-	-

**Supplementary Figure 3** Extent of protein cross-linking (as detected by SDS-PAGE gels with silver staining) induced on incubation of mixtures containing BLP or Kyn-bound to BLP (each ca. 6  $\mu\text{M}$  in protein), with Cu(II) (10  $\mu\text{M}$ ) or Fe(III) (10  $\mu\text{M}$ ), as indicated by the grid, under aerobic conditions in PBS at 37  $^{\circ}\text{C}$  for 24 h in the dark. Lane 5 in the middle of the gel contained molecular mass markers. It should be noted that this gel has been stained for a much longer time compared to other figures in order to detect any potential Kyn-mediated cross-links.



**Supplementary Figure 4** Effect of varying concentrations of Cu(II) and Fe(III) on 3OHKyn oxidation as detected by UV/Visible spectroscopy. 3OHKyn (250  $\mu\text{M}$ ) was incubated at 37 $^{\circ}$  in the dark with the indicated concentrations of Cu(II) (panels A and B) or Fe(III) (panels C and D) in phosphate-buffered saline (pH 7.4) for 1 h (panel A), 2 h (panels B, C) or 24 h (panel D).

**Supplementary Table** Levels of  $E^+$  (from HPLC analysis) and total fluorescence detected on oxidation of DHE (200  $\mu$ M) after incubation at 37° in the dark with 3OHKyn or 3OHAA (both 10  $\mu$ M) in phosphate-buffered saline (pH 7.4) without or with 5  $\mu$ M Cu(II) for 1 h.

<i>Reaction system</i>	<i><math>E^+</math> (arbitrary units)</i>	<i>Total fluorescence (arbitrary units)</i>
DHE	6.5	7.1
DHE + Cu(II)	8.3	8.4
DHE + 3OHKyn	12.9	12.9
DHE + 3OHKyn + Cu(II)	22.3	22.3
DHE + 3OHAA	8.4	9.1
DHE + 3OHAA + Cu(II)	16.4	16.4